

CELL CULTURE SYSTEM AND CELL MASS PRODUCTION METHOD USING SAME

TECHNICAL FIELD

[0001] The present invention relates to a cell culture system that makes it possible to repeat division of cell aggregates obtained by growing cells in suspension culture, and further suspension culture thereof, in a closed system, and a method for producing cell aggregates by using the cell culture system.

BACKGROUND ART

[0002] In recent years, a cell culture method (also called a suspension culture method) has been developed in which various cells such as pluripotent stem cells and the like are suspended in a liquid medium and three-dimensionally grown into a cell aggregate (e.g., patent document 1 and the like). In addition, a liquid medium for preferably performing the suspension culture method and a production method thereof have also been developed (e.g., patent document 1 and the like).

[0003] In suspension culture method, the undifferentiated state of pluripotent stem cells may decrease as the cell aggregate grows larger. For example, non-patent document 1 suggests that the undifferentiated state of large cell aggregates of 150 μm or more may decrease.

[0004] On the other hand, in the culture method of pluripotent stem cells described in patent document 1, pluripotent stem cells are suspension cultured until they become large cell aggregates having an average diameter of about 200-about 300 μm , the obtained large cell aggregates are divided into smaller cell aggregates having an average diameter of about 80 to about 120 μm , after which suspension culture is further continued to maintain and amplify the pluripotent stem cells.

DOCUMENT LIST

Patent Documents

[0005] patent document 1: WO 2013/077423

[0006] patent document 2: WO 2016/163444

Non-Patent Document

[0007] non-patent document 1: Andreas Elanzew et al., "A reproducible and versatile system for the dynamic expansion of human pluripotent stem cells in suspension", *Biotechnology Journal*, 2015, 10, 1589-1599.

SUMMARY OF INVENTION

Technical Problem

[0008] The present inventors studied in detail the culture method of a pluripotent stem cell described in patent document 1 and found that the method includes the following problem to be improved.

[0009] In the aforementioned culture method, the step of dividing the cell aggregate and the step of further culturing the divided cell aggregate are performed using a container and a dividing instrument separate from each other. Therefore, the cell aggregate and a liquid medium containing same come into contact with the outside air when they are sent to the division step, and the cell aggregate divided in the

division step and the liquid medium containing same come into contact with the outside air when they are sent to a culture container in the next stage for further culture. Therefore, when the step of dividing the cell aggregate and the step of further culturing the divided cell aggregate are repeated, the cell aggregate and the liquid medium come into contact with the outside air many times and may be contaminated.

[0010] The present invention aims to provide a cell culture system that can solve the above-mentioned problems, and make it possible to perform division of cell aggregates and further suspension culture of the divided cells in a closed system, and a method for producing cell aggregates by using the cell culture system.

Solution to Problem

[0011] The main constitution of the present invention is as follows.

[1] A cell culture system for dividing and subculturing a cell aggregate, comprising at least:

[0012] a divider for dividing a cell aggregate to be divided which is supplied together with a liquid medium from a supply source into smaller cell aggregates; and

[0013] a first container for suspension culturing the cell aggregates divided by the divider; wherein,

[0014] the divider has an inlet, an internal dividing conduit, and an outlet, the inlet is constituted such that the cell aggregate to be divided and the liquid medium are received from the supply source into the dividing conduit, the dividing conduit is provided with a mesh structure to divide the cell aggregate to be divided, the cell aggregate to be divided is divided when passing through the mesh structure together with the liquid medium, and the outlet is connected to the first container so as to deliver the divided cell aggregates to the first container, and

[0015] the first container has a constitution for receiving the divided cell aggregates and the liquid medium and sending out the cell aggregates suspension cultured in the first container.

[2] The cell culture system according to [1], wherein the first container is a flexible cell culture bag.

[3] The cell culture system according to [1] or [2], wherein the mesh structure is a mesh woven with wire.

[4] The cell culture system according to [1] or [2], wherein the mesh structure is a porous film with many through-holes disposed on the film surface, the mesh structure comprises many through-holes penetrating the predetermined region in the film thickness direction, and a beam part serving as a partition between the through-holes,

[0016] the through-holes have an opening shape of a size permitting passage of the aforementioned smaller cell aggregates,

[0017] and the beam part is a remainder after subtracting the through-hole from the main body part in the predetermined region, is a part that cuts the cell aggregates to be divided, and is integrally connected to form a network.

[5] The cell culture system according to [4], wherein

[0018] a cross-sectional shape in the perpendicular longitudinal direction of the aforementioned beam part is a rectangle, or two corners on the inlet side of the rectangle have a round shape.